Controlling the Type I and Type II Errors in Mapping Quantitative Trait Loci

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ABSTRACT

Although the interval mapping method is widely used for mapping quantitative trait loci (QTLs), it is not very well suited for mapping multiple QTLs. Here, we present the results of a computer simulation to study the application of exact and approximate models for multiple QTLs. In particular, we focus on an automatic two-stage procedure in which in the first stage "important" markers are selected in multiple regression on markers. In the second stage a QTL is moved along the chromosomes by using the preselected markers as cofactors, except for the markers flanking the interval under study. A refined procedure for cases with large numbers of marker cofactors is described. Our approach will be called MQM mapping, where MQM is an acronym for "multiple-QTL models" as well as for "marker-QTL-marker." Our simulation work demonstrates the great advantage of MQM mapping compared to interval mapping in reducing the chance of a type I error (*i.e.*, a QTL is indicated at a location where actually no QTL is present) and in reducing the chance of a type II error (*i.e.*, a QTL is not detected).

THE advent of maps of molecular markers enables geneticists to detect and map individual loci affecting quantitative traits (*cf.* PATERSON *et al.* 1988). In the ideal case all genetic variance of the trait is explained by detected quantitative trait loci (QTLs). In practice a number of QTLs may be missed (a type II error) and at the same time a number of false positives may occur, indicating QTLs at map positions (or regions) where actually no QTLs are present (a type I error). The actual balance between the cost of false positives and the benefit of detected QTLs depends on the aim of the experiment (*e.g.*, map-based cloning or introgression breeding). Nevertheless, one often strives for keeping at least the chance of a type I error below 5%. Therefore, the QTL mapping method used should keep the chance of a type I error below 5%, but at the same time it should minimize the chance of a type II error. The interval mapping method (LANDER and BOTSTEIN 1989) is widely used, but it is now generally recognized that the chance of a type I or a type II error is higher in interval mapping than it is in simultaneous mapping of multiple QTLs (*cf.* HALEY and KNOTT 1992; MARTINEZ and CURNOW 1992; JANSEN 1993b). This has motivated theoretical research for multiple QTL mapping methods. Recently, JANSEN (1992, 1993b) and JANSEN and STAM (1994) developed a unifying framework of exact and approximate models for multiple QTLs, from now on called MQM mapping. MQM is an acronym for "multiple-QTL models" but also for "marker-QTL-marker" (which reflects the insertion of QTLs between markers on the genetic linkage map). The framework includes interval mapping and regression on markers (COWEN 1989; STAM 1991; RODOLPHE and LEFORT 1993; ZENG 1993) and also includes their "hybrid" in which the phenotype is regressed on a single putative QTL in a given marker interval, and at the same time on a number of markers located elsewhere on the genome. The rationale behind using markers as cofactors is that these markers will eliminate the major part of the variation induced by nearby QTLs. Some simulation work (JANSEN 1993b) and a practical application (JANSEN and STAM 1994) indicated that the MQM mapping method is computationally feasible and substantially more powerful than interval mapping. For the present paper a computer simulation study was set up to study more thoroughly the chances of a type I or II type error in MQM mapping, and to compare MQM mapping with interval mapping. A number of QTL configurations were studied by simulation, covering the most relevant multiple-QTL configurations; the results are presented and discussed.

STATISTICAL MODELS FOR MQM MAPPING

In this section statistical aspects of MQM mapping are summarized. For more details see JANSEN (1992, 1993b) and JANSEN and STAM (1994). Further refinements to MQM mapping are proposed, concerning the testing for the presence of a putative QTL, and concerning the parameter estimation for the case that many marker cofactors are used.

The framework: We restrict ourselves to backcross progenies, but the same method applies to other inbred or outbred progenies. Furthermore, we assume a normally distributed environmental error. The general model in MQM mapping is $Y = m + x_i a_i + E$, where Y is the phenotypic trait, m is the mean, a_i are the allele substitution effects of individual loci and E is the (environmental) error; the summation is over all loci affecting the trait. The x_i are indicator variables specifying

the genotype. In a backcross progeny they can take two values: 0 or 1. The loci in the above expression can be one or more QTLs, but–as an approximation–markers can be used as well. Therefore, the model includes interval mapping (LANDER and BOTSTEIN 1989), but also exact models for multiple QTLs (JANSEN 1992, 1993b; JANSEN and STAM 1994), multiple regression on markers (COWEN 1989; STAM 1991; RODOLPHE and LEFORT 1993; ZENG 1993), and the hybrid between interval mapping and multiple regression on markers (JANSEN 1993b) in which marker cofactors are selected prior to the analyses considering a QTL in each interval in turn. Parameter estimation is based on the simultaneous distribution of the genotype and phenotype; the core of our method is completion of any missing genotypic (QTL and marker) information, which is embedded in a general and simple EM algorithm to obtain maximum likelihood estimates of the model parameters (JANSEN and STAM 1994). In the case of exact models for multiple QTLs, this procedure makes simultaneous estimation of QTL positions possible.

Preselection of marker cofactors: Markers can be used in the regression to take over the role of nearby QTLs. STAM (1991) demonstrated that in multiple regression the effect of a QTL is absorbed only by its flanking markers, at least if the progeny size is large; other markers are then redundant. Since the locations of the QTLs are generally unknown, the question is which markers have to be used as cofactors in MQM mapping. A standard regression selection procedure can be used to select the "important" markers. One such procedure is backward elimination of marker cofactors in multiple regression of the phenotype on the markers. JANSEN (1993b) minimized Akaike's information criterion, AIC $= -2(\mathscr{L} - k)$, where $\mathscr{L}$ is the log-likelihood and k is the number of free parameters in the model. Here, we minimize $-2(\mathscr{L} - 3k)$, i.e., a more stringent penalty for the number of free parameters is used. In "ordinary" regression with adequate degrees of freedom to estimate σ^2, a penalty of k is equivalent to the use of (about) the 16% point of the F test for the comparison of two nested models, which differ only by the inclusion of one free parameter; a penalty of $3k$ is equivalent to the use of (about) the 2% point (MCCULLAGH and NELDER 1989). At each step of the backward elimination process a marker is dropped, namely the marker which gives the largest decrease of the criterion; the process is stopped when no further reduction of the criterion can be achieved. In the next stage (the actual mapping stage), the selected markers will be used as cofactors. For proper marker selection a reasonable number of recombinants between flanking markers is required (the larger the QTL effect, the fewer recombinants are required). Because of the near collinearity of closely linked marker cofactors, it makes little sense to use a very dense map in a progeny of, say, 100 individuals.

Very recently, ZENG (1994) presented a simulation study in which all markers were used as cofactors, except for the markers flanking the interval under study. He, however, also suggested preselecting the markers which explain most of the genetic variation in the genome.

Testing for the presence of a putative QTL: In MQM mapping at each map location the log-likelihood $\mathscr{L}_1$ for a single QTL in a given interval can be calculated and compared with the log-likelihood $\mathscr{L}_0$ of no QTL in the given interval, using in both models the same set of marker cofactors (or the same set of QTLs in other intervals when exact models for multiple QTLs are used). The likelihood-ratio test statistic for the presence of a putative QTL in a given interval is then expressed as the maximum of $2(\mathscr{L}_1 - \mathscr{L}_0)$ over the interval. The distribution of the test statistic for the presence of a QTL in a specific interval is not exactly known. However, when no QTLs are segregating, the asymptotic distribution is expected to be between the χ_1^2 and χ_2^2 distribution (TITTERINGTON et al. 1985). The latter distribution is justified by the difference in the number of parameters (one for the allele substitution effect a of the putative QTL, and one for the location of the QTL in the marker interval). The former is justified by the fact that the null hypothesis is defined by the single constraint $a = 0$. LANDER and BOTSTEIN (1989) and VAN OOIJEN (1992) simulated the distribution of the test statistic. Based on extensive simulations these authors published appropriate thresholds for the test statistic so that the chance of a false positive occurring anywhere on the genome is at most 5% (still under the assumption that no QTLs are segregating). We here suggest that these thresholds are also suitable for MQM mapping: they can be used when no QTLs are segregating, since in that case it is expected that no or only a very few markers will be selected in MQM mapping. Moreover, these thresholds can also be used when QTLs are segregating, the effects of which are eliminated by marker cofactors in MQM mapping. One condition is, however, that the number of degrees of freedom for estimating σ^2 is large enough (see below).

Marker cofactors should not replace the putative QTL in the interval of current interest. It was decided to study a simple approach to prevent this: for a given interval all selected markers are used as cofactors, except the ones flanking the interval of current interest. We expect that this approach applies well if marker selection is properly based on reasonable numbers of recombinants between flanking markers (see above). Otherwise, a general (but more computer intensive) selection approach can be used (JANSEN 1993b): starting from the single-QTL model using all selected markers, it is assessed which nearby markers still may be dropped (those markers previously explained the effect of the QTL), and which markers cannot be dropped (these markers possibly absorb the effects of other QTLs on the current chromosome).

When the number of marker cofactors is large: In ordinary regression the number of parameters estimated from the data should not be too large when maximum likelihood is used. Asymptotic relations such as the χ^2 approximations do not necessarily hold in the case of large numbers of parameters. The main reason for this is the bias of the maximum likelihood estimate of the residual variance. The usual bias adjustment of the estimate of the variance is to multiply the estimate by $N/(N-p)$, where N is the number of individuals and p is the number of free parameters used for modeling the relation between the mean and explanatory variables. When comparing a sequence of (nested) models we have the option of using a common estimate of variance for all models in the sequence, or using separate estimates derived from the fit of each model in turn (Mc-CULLAGH and NELDER 1989). In "ordinary" regression analysis a single estimate of the variance obtained from the most complex model is usually considered. This estimate of the variance is used for all models in the sequence, which at the same time guarantees that the test statistic takes only positive values (*cf.* HALEY and KNOTT 1992). This property does not hold if for each model a separate bias-adjusted estimate of the variance is used. Here, we deal with mixture models instead of "ordinary" regression models, because of missing QTL and marker observations (it is quite common that a small proportion of the marker data are missing). Variable selection and bias adjustment of the maximum-likelihood estimate of the residual variance in mixture models is an area open to research, probably because mixture models with many parameters did not occur before. Mixture analysis can be viewed as "ordinary" regression with missing values for one or more factors (JANSEN 1992, 1993a). Therefore, it is natural to adapt the approach for variable selection and bias adjustment in regression models to the case of mixture models. In MQM mapping with complete linkage maps we propose the use of the following heuristic three-step procedure: (1) obtain maximum-likelihood estimates for the most complex model (usually the model for regression of phenotype on all markers); (2) adjust the estimate of the residual variance for bias; and (3) obtain maximum-likelihood estimates in the sequence of models (in the models for regression of phenotype on subsets of the markers during the selection process, or in single-QTL and no-QTL models with selected marker cofactors), keeping the variance fixed at the value obtained from step 2.

Following this approach, the distribution of the test statistic for the presence of a QTL in a specific interval is expected to be between the $F_{1,\text{d.f.}}$ and $2F_{2,\text{d.f.}}$ distribution rather than between the χ_1^2 and χ_2^2 distribution, where d. f. are the degrees of freedom for estimating σ^2 (HALEY and KNOTT 1992). Therefore, appropriate thresholds for an entire genome should also be functions of the number of residual degrees of freedom. Of course, F and χ^2 distributions are closely related if the number of residual degrees of freedom is large.

SIMULATIONS

For a number of specified configurations of QTLs and QTL effects, we studied the distribution of the test statistic for the presence of a putative QTL. These configurations include no QTL, a single QTL or two QTLs, the two QTLs being unlinked, linked in repulsion (*i.e.*, with opposite sign effects) or linked in coupling phase (*i.e.*, with equal sign effects). Furthermore, we considered small and large numbers of markers. In MQM mapping with many cofactors, a common and bias-adjusted estimate of the variance was used for all models according to the procedure described above. See Figures 1–9 for the description of the various settings. Putative QTLs are detected via the following procedures: (a) by MQM mapping using (selected) markers as cofactors, (b) by MQM mapping with exact models for multiple QTLs, and (c) by interval mapping. In all cases we simulated by computer and according to the Mendelian segregation rules the genotypes and phenotypes of 100 individuals as if they had been produced by back-crossing F_1 individuals to one of the parents. For each genetic setting 500 simulations were run. Marker distances were assumed to be known and to be equal to the values used for simulation. For each genetic setting we plotted the simulated distribution of the test statistic in a given interval (the maximum of $2(\mathscr{L}_1 - \mathscr{L}_0)$ over all map locations in the given interval). The distributions turned out to be markedly skewed. To have a better presentation we plotted the square root of the test statistic. Two types of simulation were run: (a) simulations concerning configurations with no QTL in the interval of interest, aiming at a study of the type I error, and (b) simulations with a QTL in the interval of interest, aiming at a study of the type II error. They are dealt with in the next two sections, respectively.

Type I error

The distribution of the test statistic for the presence of a putative QTL was simulated for a given marker interval, in which actually no QTL is located. When no QTLs are segregating or when the effects of QTLs are sufficiently eliminated, this distribution is expected to be between the χ_1^2 and χ_2^2 distribution. If the number of degrees of freedom for estimating σ^2 is small, this distribution is expected to be between the $F_{1,\text{d.f.}}$ and $2F_{2,\text{d.f.}}$ distribution.

We successively considered the following situations. A single QTL is located on the same chromosome as the interval under study, or on another chromosome; or two QTLs in coupling phase are located on the same chromosome, one on either side of the marker interval of interest. We also considered how the distribution of the test statistic is affected by the number of free parameters
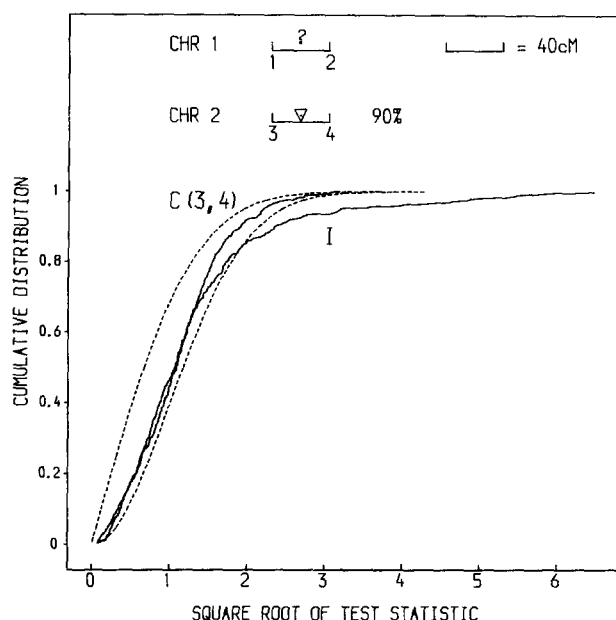
FIGURE 1.—A study of the type I error in case a single QTL is present on another chromosome. Backcross progenies of 100 individuals were simulated. Markers are numbered from the left to the right on each chromosome. The question mark (?) indicates the marker interval under study in which the likelihood for the presence of a putative QTL is assessed. The symbols ∇ and $\triangle$ indicate the position of a QTL with an effect of positive and negative sign, respectively. The percentage (beside the chromosome) indicates what percentage of the expected total phenotypic variance is attributable to the expected (simultaneous) genetic variance of the QTLs on the given chromosome. The finely dashed curves indicate the χ_1^2 distribution (left) and the χ_2^2 distribution (right). The coarsely dashed curves indicate the $F_{1,17}$ distribution (left) and the $2F_{2,17}$ distribution (right). The solid curves indicate:

I, interval (I) mapping;

C(3, 4), MQM mapping with markers 3 and 4 as cofactors (C). Analogous definition for other sets of marker cofactors;

S, MQM mapping with selected (S) markers as cofactors (but markers flanking the interval under study are not used as cofactors, even if they were selected);

E, MQM mapping with exact (E) models for multiple QTLs;

A, MQM mapping with two adjustments (A): (1) the estimate of the variance in the most complex model is adjusted for bias and, (2) the variance in any other model is fixed at the value obtained from the most complex model;

IA, combination of I and A;

CA, combination of C and A;

SA, combination of S and A.

to be estimated from the data. Finally, we studied the maximum value of the test statistic in an entire genome in absence of segregating QTLs. See Figures 1–5 for the description of the QTL configurations.

A single QTL present on another chromosome: First, we studied the case that no QTL is present in the interval of interest, while a single QTL is present on another chromosome (Figure 1). In interval mapping the distribution of the test statistic may be affected by an unlinked major QTL when the marker interval 1–2 is wide (curve I): the curve deviates from the χ^2 distributions. In

MQM mapping, the distribution of the test statistic is unaffected by the QTL when the markers 3 and 4 are used as cofactors (curve C(3, 4)). It is of course generally unknown where the QTLs are and therefore one does not know which markers should be used as cofactors to absorb them. The QTL has, however, a major effect and when marker selection was applied, markers 3 and 4 were selected in almost all simulations (not shown). Therefore, the curve for MQM mapping with selected markers as cofactors almost coincides with the curve C(3, 4). As the expected genetic variance of the QTL represents 90% of the expected phenotypic variance, these simulations show the maximal influence of a single QTL on the type I error in an interval on another chromosome.

A single QTL present on the same chromosome: Next, we studied the case that no QTL is present in the interval of interest, while a single QTL is present on the same chromosome (Figure 2, a–d). Both in interval mapping and in MQM mapping the presence of a major QTL in marker interval 2–3 has a very strong influence on the test statistic in marker interval 1–2, even when the markers 2 and 3 are used as cofactors (curves I and C(2, 3), respectively, in Figure 2a). We also considered the case that a major QTL is not in marker interval 2–3, but in marker interval 3–4 (Figure 2b). In interval mapping, the test statistic in marker interval 1–2 is still highly affected by the QTL (curve I in Figure 2b); in MQM mapping however, it is unaffected when the markers 3 and 4 are used as cofactors (curve C(3, 4) in Figure 2b). In practice it is not *a priori* known that, for instance, a QTL is located in marker interval 3–4 and that therefore marker 3 and 4 should be used as cofactors to absorb the effect of the QTL. When marker selection was applied, in almost all simulation runs the two markers flanking the QTL were selected: marker 2 and 3 in Figure 2a, and marker 3 and 4 in Figure 2b. In the first case the corresponding curve S deviates even more from the χ^2 distributions than that curve C(2, 3) deviates from them, and in the second case curve S coincides with curve C(3, 4) (S curves are not plotted). As the expected genetic variance of the QTL forms the major part of the expected phenotypic variance (90%), these simulations show the maximal influence of a single QTL on the type I error in another interval on the same chromosome.

We also simulated the same configurations with a QTL with a much smaller effect (Figure 2, c and d). In interval mapping, the test statistic is still highly affected by the presence of a QTL in marker interval 2–3 (curve I in Figure 2c), or by the presence of a QTL in marker interval 3–4 (curve I in Figure 2d). In neither case is the test statistic influenced in MQM mapping when the markers 2–3 or 3–4 are used as cofactors (curves C(2, 3) and C(3, 4) in Figure 2, c and d, respectively). When marker selection was applied, in many simulation runs
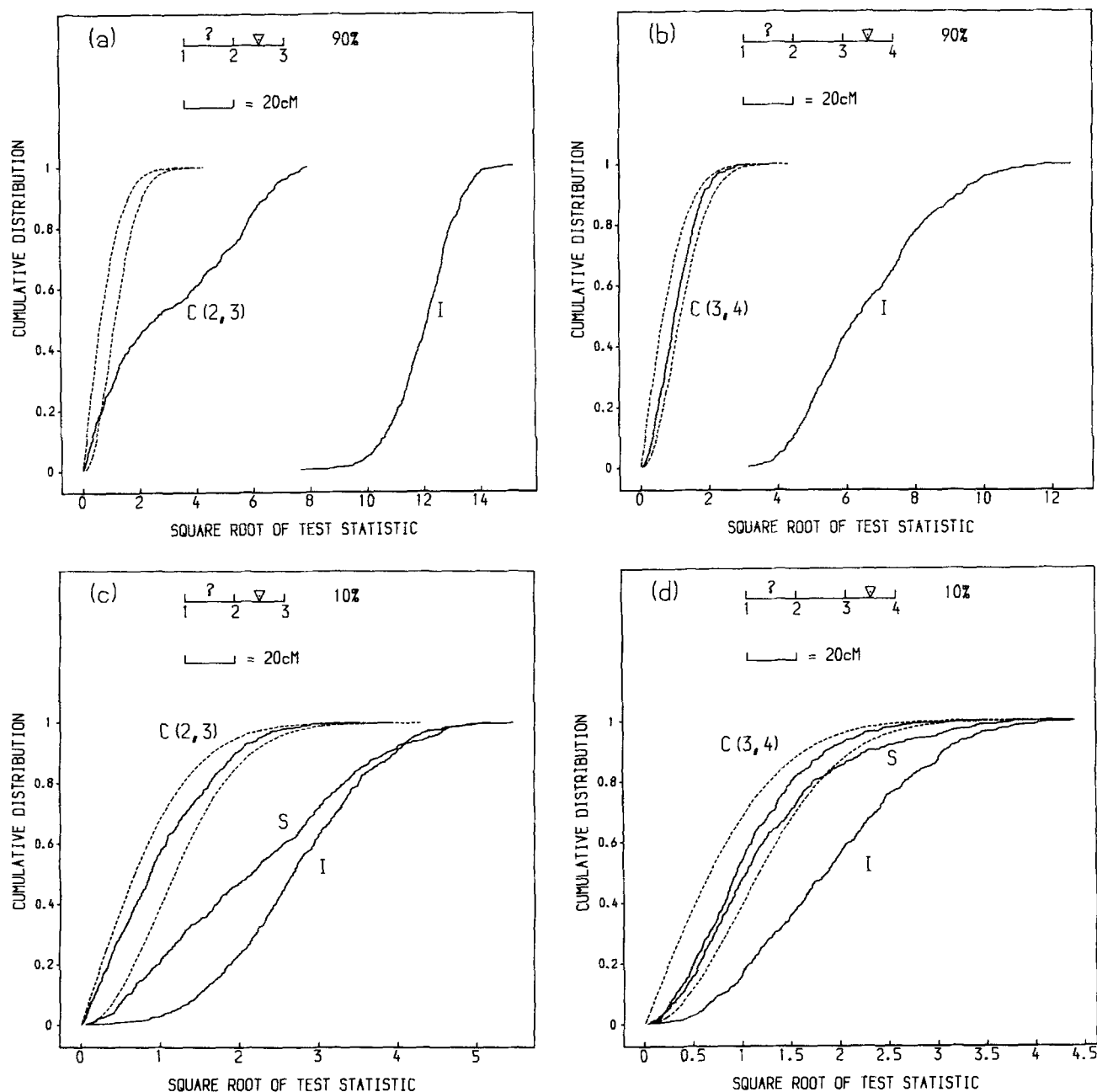
FIGURE 2.—A study of the type I error in case a single QTL is present on the same chromosome (see Figure 1 legend).

only one of the two markers flanking the QTL was selected (marker 2 or 3 in Figure 2c, marker 3 or 4 in Figure 2d). Since markers flanking the interval of interest are not used as cofactors when applying marker selection, this seriously affects the test statistic in the case of a QTL in the interval adjacent to the interval of interest (curve S in Figure 2c). However, when an additional marker between the interval of interest and the QTL is available, the test statistic corresponding tomarker selection is hardly affected (curve S in Figure 2d).

Two linked QTLs in coupling phase: Then, we studied the case of two linked QTLs in coupling phase (*i.e.*, with effects of equal size and equal sign; see Figure 3).

It is well known that in interval mapping the test statistic in this case will often be at its maximum in one of the intermediate intervals (MARTINEZ and CURNOW 1992). This can lead to the detection of a single QTL in the wrong interval (a type I error). Therefore, we studied the effect of both QTLs on the test statistic in the intermediate marker interval 4–5. The effect of the second QTL in marker interval 6–7 on the test statistic for the first QTL in marker interval 2–3 is dealt with in the next section (aiming at a study of the type II error).

In MQM mapping, the distribution of the test statistic for the presence of a putative QTL in marker interval 4–5 is unaffected by the two QTLs when the markers 2, 3, 6 and 7 are used as cofactors (curve C(2, 3, 6, 7)), and
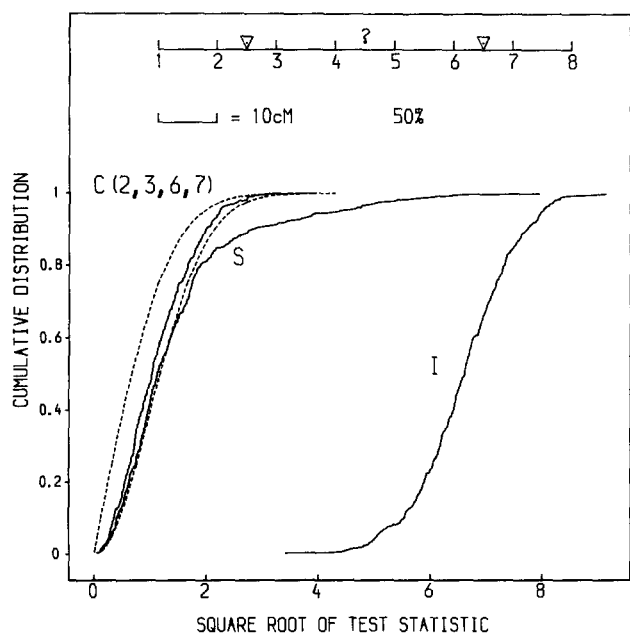
FIGURE 3.—A study of the I type error at an interval between two QTLs in coupling phase (see Figure 1 legend).

only slightly affected when selected markers are used (curve S). In interval mapping, the test statistic takes very large values (curve I).

The effect of the number of cofactors: Furthermore, we studied the effect of the number of cofactors on the type I error in MQM mapping with 80 marker cofactors distributed over 16 other chromosomes (Figure 4). This figure shows that the test statistic is seriously affected (curve C(3–82)). Therefore, it is clear that the number of redundant cofactors should not be too large in maximum likelihood estimation. Bias adjustment of the estimate of the variance could be a solution to this problem and therefore we reanalyzed the case, using the bias adjustment procedure described above. The distribution of the test statistic for the case of 80 cofactors with the bias adjustment is between the $F_{1,17}$ and $2F_{2,17}$ distribution (curve CA(3–82)), and so is the distribution of the test statistic when marker selection is combined with bias adjustment (curve SA). This confirms that the bias adjustment works.

The maximum value of the test statistic in an entire genome: Finally, we studied the type I error in a genome with 40 markers distributed over 8 chromosomes (Figure 5). In MQM mapping, we applied the variable selection and bias adjustment procedure, developed above for the case of many marker cofactors (curve SA). No or only a very few markers were selected (394 times no markers, 72 times one marker, 16 times two markers, 9 times three markers, 8 times four markers and only once five markers; together 500 simulation runs). The same case was reanalyzed using the interval mapping method (curve I). The two distributions of the maximum value of the test statistic in the entire genome are very close to each other. This demonstrates that the results by LANDER and BOTSTEIN (1989) and VAN OOIJEN
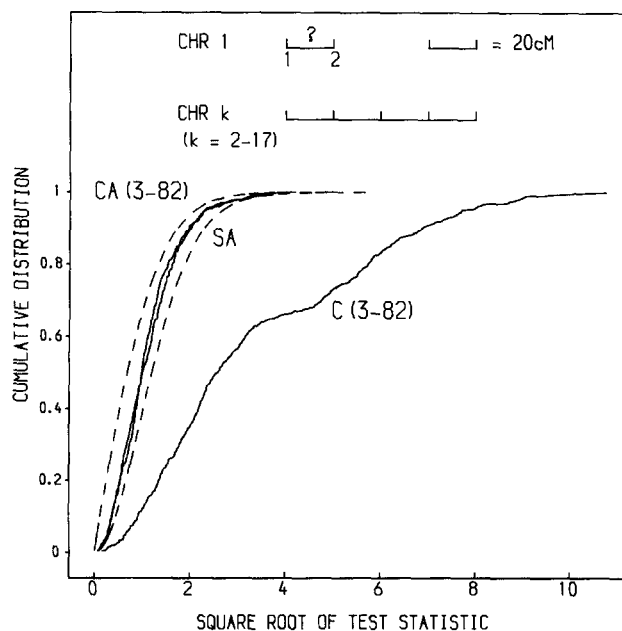

FIGURE 4.—A study of the type I error in case of many marker cofactors (see Figure 1 legend).
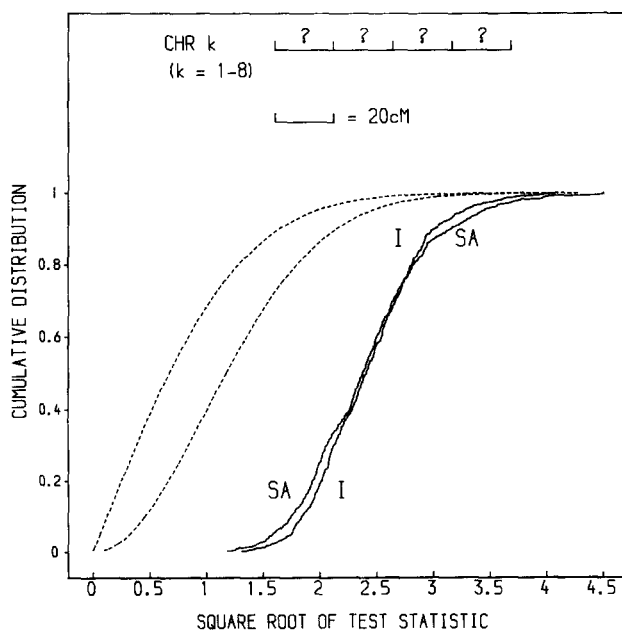

FIGURE 5.—A study of the type I error in an entire genome (see Figure 1 legend).

(1992) can be generally used to choose a threshold for the test statistic such that the probability of a type I error is about 5%. Of course, the methods differ with respect to the estimation of σ^2, so that at least small differences can be expected. Moreover, the thresholds from interval mapping are less appropriate for MQM mapping if there are only a few degrees of freedom for estimating σ^2. Further simulation should reveal the thresholds for these situations (see also discussion below).

Type II error

The distribution of the maximum value of the test statistic for the presence of a putative QTL was simulated
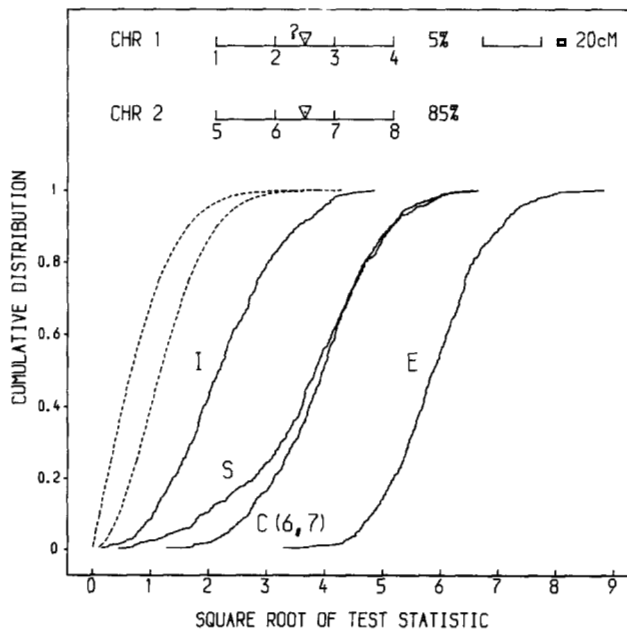
FIGURE 6.—A study of the type II error in case of two unlinked QTLs (see Figure 1 legend).

FIGURE 7.—A study of the type II error in case of two linked QTLs in repulsion phase (see Figure 1 legend).

in an interval in which a QTL is actually segregating. We successively considered the following situations: another QTL is also segregating and the two QTLs are either unlinked, linked in repulsion, or linked in coupling phase. See Figures 6–9 for the description of the QTL configurations. We studied the effect which the second QTL has on the test statistic for the presence of the first QTL. We say that the QTL is detected if the test statistic exceeds the threshold at a significance level of 5% for a 1000-cM genome. This means that we assume the simulated intervals to be part of a large genome. The value of this threshold is $2.4 \cdot 2 \cdot \ln(10) \approx 11.05$ (LANDER and BOTSTEIN 1989; LOD threshold = 2.4, see their Figure 4). The square root of the threshold is equal to 3.32.

Finally, we also considered the effect of bias adjustment of the estimate of the variance and the effect of a common estimate of the variance in sequences of models (see Figure 9).

Two unlinked QTLs: First, we studied the case of two unlinked QTLs (Figure 6). In interval mapping, the first QTL in marker interval 2–3 is detected with a chance of 0.14 (curve I). In MQM mapping with markers 6 and 7 as cofactors the first QTL is detected with a chance of 0.74 (curve C(6, 7)). In general the locations of the QTLs are unknown, so that markers to be used as cofactors should be selected. In some cases marker 1 or marker 4 may be selected and used as cofactor. The markers 1 and 4 are linked to the first QTL and can also (partially) absorb the effect of this QTL. As a consequence, the test statistic takes the smaller values in these cases (lower tail of curve S in Figure 6). Nevertheless, the chance of detecting the first QTL is still 0.70 when selected markers are used. This demonstrates that QTLs can be detected more powerfully by MQM mapping than by interval mapping (the chance of detection of the
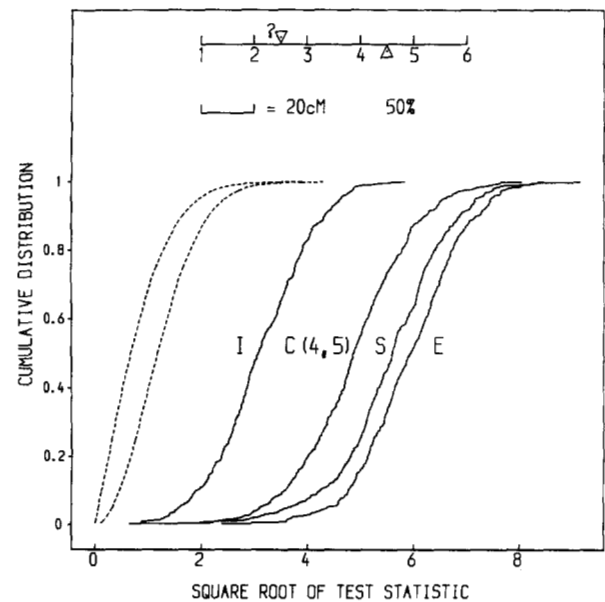
first QTL is 0.70 vs. 0.14, respectively). As the expected genetic variance of the QTL forms the major part of the expected phenotypic variance (90%), these simulations show the maximal increase in power.

MQM mapping with marker cofactors was also compared to MQM mapping with exact models for two QTLs. To that order, a putative QTL, or no QTL, was fitted in marker interval 2–3, while in either case a second QTL was fitted in marker interval 6–7 (curve E in Figure 6). It is clear from Figure 6 that the curves C(6, 7) (or curve S) and E are still rather different. This means that a proportion of the genetic variation of the second QTL could not be eliminated by the marker cofactors, due to recombinants between marker 6 (or 7) and the second QTL. Thus, MQM mapping with exact models for multiple QTLs is sometimes much more powerful than MQM mapping with marker cofactors. It is clearly beneficial to use exact models for those putative QTLs that have a major effect on the trait (and the corresponding marker cofactors can be dropped).

Two linked QTLs in repulsion phase: Next, we studied the case of two linked QTLs in repulsion phase (with effects of equal size but opposite sign; see Figure 7). In interval mapping, the first QTL in marker interval 2–3 is detected with a chance of only 0.42 (curve I). In MQM mapping with markers 4 and 5 as cofactors the first QTL is detected with a chance of 0.93 (curve C(4, 5)). When only selected markers are used in MQM mapping (excluding the markers 2 and 3, which flank the interval under study), the chance of detecting the first QTL increases even to 0.97 (curve S). The increase is due to the fact that marker 4 sometimes partially absorbs the large effect of the first QTL, while marker 5 absorbs the large effect of the second QTL in marker interval 4–5; the value of the test statistic for the presence of the first QTL
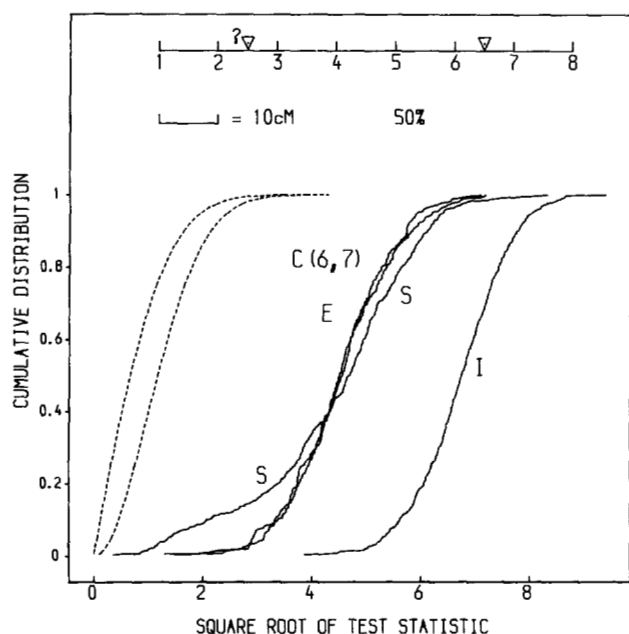
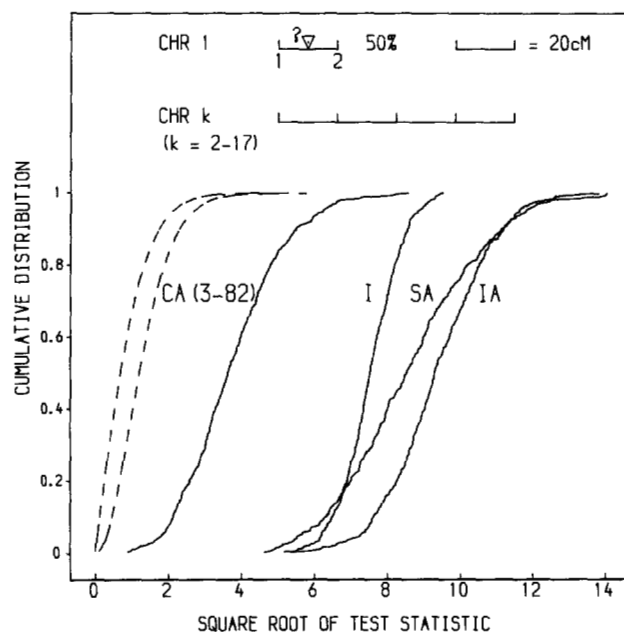FIGURE 8.—A study of the type II error in case of two linked QTLs in coupling phase (see Figure 1 legend)

FIGURE 9.—A study of the type II error in case of many marker cofactors (see Figure 1 legend)

then increases by dropping marker 4 or 5. Our simulations demonstrate that linked QTLs in repulsion phase can be detected and separated much more powerfully by MQM mapping with marker cofactors than by interval mapping.

MQM mapping with marker cofactors was also compared to MQM mapping with exact models for two QTLs. To that order, a putative QTL, or no QTL, was fitted in marker interval 2–3 while in either case a second QTL is fitted in marker interval 4–5 (curve E). It is clear from Figure 7 that curve S and curve E are only slightly different, i.e., MQM mapping with marker cofactors is almost as powerful as MQM mapping with exact models for multiple QTLs, when QTLs are in repulsion phase.

Two QTLs in coupling phase: Then, we studied the case of two linked QTLs in coupling phase (with effects of equal size and equal sign; Figure 8). In interval mapping, the test statistic for the presence of the first QTL in marker interval 2–3 exceeds the threshold with a chance of 1.00 (curve I). In MQM mapping with markers 6 and 7 as cofactors the test statistic for the first QTL exceeds the threshold with a chance of 0.92 (curve C(6, 7)). Thus, in contrast to the results for the previous configurations, MQM mapping now leads to smaller values for the test statistic than interval mapping does. The reason for this is that the effect of the first QTL, but also the major part of the effect of the second QTL in marker interval 6–7, are absorbed when interval mapping of a single putative QTL is carried out in marker interval 2–3. In MQM mapping, the effect of the second QTL and that of the major part of the first QTL are absorbed by the marker cofactors 6 and 7; the test statistic for marker interval 2–3 gives the likelihood for the presence of mul-

tiple linked QTLs, one being located in marker interval 2–3, the other being located nearby markers 6 and 7. When only selected markers are used in MQM mapping (excluding markers 2 and 3, which flank the interval under study), the test statistic for the presence of a QTL in marker interval 2–3 decreases slightly (the chance of detection of the QTL is 0.81; curve S). The reason for this is that markers 1 or 4 were selected (as well) in some simulation runs. The upper tail of curve S exceeds the upper tail of curves E and C(6, 7) slightly. The reason for this is that either marker 6 or marker 7 was selected in a number of simulations (rather than selecting markers 6 and 7 simultaneously), thereby absorbing slightly less variation induced by the QTLs.

MQM mapping with marker cofactors was also compared to MQM mapping with exact models for two QTLs. To that order, a putative QTL, or no QTL, was fitted in marker interval 2–3 while in either case a second QTL was fitted in marker interval 6–7 (curve E). It is clear from Figure 8 that curve C(6, 7) and curve E are very close; however, the lower tail of curve S deviates from them. Thus, not unexpectedly, MQM mapping with selected marker cofactors is not always as powerful as MQM mapping with exact models for multiple QTLs, when QTLs are in coupling phase.

The effect of the number of cofactors: Finally, we will discuss the effect on the type II error of two changes to MQM mapping, namely the use of a single estimate of the variance in a sequence of models and the bias adjustment of the estimate of the variance (Figure 9). First, the test statistic for the presence of a putative QTL in marker interval 1–2 on chromosome 1 is considered without using marker cofactors for other chromosomes

(curves I and IA). Figure 9 shows the distribution for the test statistic when the usual maximum likelihood method is used (interval mapping with a separate estimate of the variance in the single and the no-QTL model; curve I) and also when the variance in the no-QTL model is fixed at the estimate from the single-QTL model (which was adjusted for bias; curve IA). In this case the bias adjustment of the estimate of the variance will be almost negligible. The simulations clearly demonstrate that the use of a common estimate of the variance can lead to a more powerful QTL detection.

Second, the test statistic for the presence of a putative QTL in marker interval 1–2 is considered when 80 markers (or a subset) on 16 other chromosomes are used as cofactors (curves CA(3–82) and SA). In such a case the estimate of the variance will be highly biased and bias adjustment is needed. The estimate of the variance in the most complex model (the model with all marker cofactors) was adjusted for bias as described above and we used this estimate as a common estimate in the subset selection procedure and in the single-QTL and the no-QTL models. The test statistic takes much smaller values when all 80 markers are used as cofactors (curve CA(3–82)) than it does when no marker cofactors are used (curve IA). This demonstrates that the 80 cofactors partially absorb the effect of the QTL in marker interval 1–2, even though these markers are located on other chromosomes. However, when preselected marker cofactors are used (curve SA), the distribution of the test statistic is much closer to the one found when no marker cofactors are used (curve IA).

DISCUSSION

The simulations presented in this report demonstrate the great advantage of MQM mapping over interval mapping in controlling the chances of type I and type II errors. The nice feature of MQM mapping is that marker cofactors are generally selected only in regions were QTLs are segregating. Because of this feature, thresholds for the test statistic, which were obtained for the case that no QTLs are segregating (LANDER and BOTSTEIN 1989; VAN OOIJEN 1992), are also suitable for MQM mapping. These thresholds can be used when no QTLs are segregating, since in that case no or only a few markers will be selected; moreover, these thresholds can still be used when there are QTLs segregating, the effects of which are eliminated by marker cofactors. One condition is, however, that the residual degrees of freedom for estimating the variance (or the dispersion parameter in generalized linear models) are adequate. In such cases, the choice of the appropriate threshold for the test statistic (so that the chance of a type I error is small, say 5%) can be made satisfactorily in MQM mapping. Further simulation work is required to reveal the appropriate thresholds for the cases in which the number of residual degrees of freedom is small. In interval mapping, the threshold for the test statistic should be used with caution. It is known that a single QTL affects the test statistic in all intervals on the same chromosome; the test statistic often exceeds the threshold in a number of intervals on either side of the QTL, although one should not "detect" multiple QTLs in this region. In MQM mapping on the other hand, the effect of a QTL diminishes rapidly when the distance between the QTL and the interval of interest increases; a QTL often affects the test statistic only in the two intervals adjacent to the one of the QTL.

The use of marker cofactors reduces the unexplained variance, so that the chance of a type II error in the case of unlinked QTLs is generally smaller in MQM mapping than in interval mapping. Our simulations also demonstrate that the detection and unraveling of the separate QTL effects in the case of linked loci is much easier in MQM mapping than in interval mapping. Linked QTLs with opposite (and mutually neutralizing) effects are worst case configurations for interval mapping: often no QTLs will be detected. Also, linked QTLs with equal sign effects are a difficult configuration for interval mapping: often a single "ghost" QTL will be detected somewhere between the two QTLs (MARTINEZ and CURNOW 1992). Again, our simulations make it clear that separation of such QTLs is much easier in MQM mapping than in interval mapping.

In our simulations the progeny size is fixed at 100 individuals, because we are involved in real experiments of that size. For such cases, VAN OOIJEN (1992) demonstrated that the chance of detecting a specific QTL is small, unless the QTL explains a large proportion of the phenotypic variance. Therefore, we considered QTL configurations for relatively high levels of heritability. Our simulations make it clear that QTLs can be mapped more powerfully by MQM mapping than by interval mapping. In some of our simulations, the gene was even of qualitative rather than quantitative nature (Figures 1, 2, a and b, and 6). We expect that a similar power improvement can be achieved when several QTLs instead of one major gene contribute to the genetic variation. Furthermore, we expect that similar results can also be obtained for smaller levels of heritability if the progeny size is larger. On the other hand, in some types of progeny such as recombinant inbred lines, the heritability can be increased at will by using more plants per line, leading to similar configurations.

In QTL-mapping experiments, large numbers of markers are commonly scored. In this paper we addressed problems concerning fitting models with many marker cofactors, and problems concerning selection of "important" marker cofactors. The maximum-likelihood estimate of the residual variance will be biased when many markers are used as cofactors; the number of parameters should not be too large, preferably less than $2\sqrt{\text{number of observations}}$ (JANSEN and STAM 1994). We propose a heuristic three-step procedure to

adjust for the bias. Our simulations demonstrate that the bias adjustment works. This makes it possible to use many markers as cofactors in MQM mapping. However, the use of redundant marker cofactors can lead to a loss of detection power. There are two causes for this loss of detection power: (a) any redundant marker which is used as a cofactor and which is located nearby the QTL can also (partially) absorb the effect of the QTL; and (b) the marker data are generally unbalanced so that the effect of a QTL can even be absorbed by redundant markers on other chromosomes, especially in small progenies. Therefore, selection of the "important" markers is beneficial. In order to exclude redundant markers, the selection criterion should be stringent, but not so stringent that important markers (those flanking the QTLs) are thereby excluded. JANSEN (1993b) proposed to maximize the log-likelihood minus the number of free parameters (k) in the model; this is equivalent to minimizing Akaike's information criterion AIC = $-2(\mathscr{L} - k)$. In general, a penalty in the range of k to $3k$ may provide plausible initial models (MCCULLAGH and NELDER 1989). In the present study, we use the more stringent penalty of $3k$. Our simulations demonstrate that (a) this penalty is stringent, since no or only a few markers are generally selected in the case of no QTLs segregating and (b) this penalty is still not too stringent, since markers are selected for those QTLs that considerably affect the test statistic in their nearby region; the effects of such QTLs are satisfactorily eliminated by selected markers. Nevertheless, we feel that it is still worthwhile to study the properties of the method for other levels of the penalty in the range from k to $3k$. In particular, we believe that the penalty should depend on the aim of the experiment. For instance, consider an experiment in which the aim is prediction of phenotypic value followed by indirect selection via markers. In the case of prediction, the penalty should be probably k rather than $3k$ (MCCULLAGH and NELDER 1989). KNOTT and HALEY (1992) discuss another situation which should be investigated in more detail: a trait with a reasonable level of heritability, which is affected by very many genes of small effect distributed throughout the genome. In general, the benefit of using a small penalty is that more variation induced by QTLs is eliminated. The cost is loss of power, since also (many) redundant markers are selected. Also, the threshold for the test statistic should become more stringent, when the penalty decreases. In order to obtain thresholds as a function of the penalty and also as a function of the residual degrees of freedom, further simulation work should be done. LANDER and BOTSTEIN (1989) and VAN OOIJEN (1992) studied the mapping of a single QTL with no markers as cofactors (equivalent to a penalty of ∞) and ZENG (1994) studied the mapping of a single QTL with all markers as cofactors, except the ones flanking the interval under study (nearly equivalent to a penalty of 0). Simulation work by ZENG (1994; his Figure 1)

demonstrated that $\chi^2_{2;\alpha/M}$ can be used as an upper bound for the $100\alpha\%$ threshold for the overall test with M intervals, unless the number of parameters is too large. It should now be obvious that the $\chi^2_{2;\alpha/M}$ relation does not hold if the number of parameters exceeds $2\sqrt{\text{number of observations}}$ (JANSEN and STAM 1994). Our work, however, makes it possible to fit properly models with many parameters. It also indicates that $2F_{2,\text{d.f.};\alpha/M}$ can be used as an upper bound, where d.f. are the degrees of freedom for estimating σ^2. Finally, we note that our selection criterion applies not only to "ordinary" regression models, assuming a normal error distribution, but also to generalized linear models (GLMs; MCCULLAGH and NELDER 1989). In comparing a sequence of GLMs, a single estimate of the dispersion parameter (σ^2 in "ordinary" regression) based on the most complex model is usually considered.

In the present report we study an automatic MQM mapping procedure. In practice the user may wish to step in interactively. Some marker cofactors could be dropped and others could be added by hand. Also, exact models for multiple QTLs could be fitted for those putative QTLs that have a major effect on the trait (and the corresponding marker cofactors may be dropped). One can still take into account the effects of less important putative QTLs by using marker cofactors. Also, exact models for two (or more) QTLs could be fitted to separate the effects of QTLs located in adjacent intervals. Such an interactive approach is possibly the most accurate and efficient way to map multiple QTLs, which is still feasible.

LITERATURE CITED

COWEN, N. M., 1989 Multiple linear regression analysis of RFLP data sets used in mapping QTLs, pp. 113–116 in *Development and Application of Molecular Markers to Problems in Plant Genetics*, edited by T. HELENTJARIS and B. BURR. Cold Spring Harbor Laboratory, Col Spring Harbor, N.Y.

HALEY, C. S., and S. A. KNOTT, 1992 A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity **69**: 315–324.

JANSEN, R. C., 1992 A general mixture model for mapping quantitative trait loci by using molecular markers. Theor. Appl. Genet **85**: 252–260.

JANSEN, R. C., 1993a Maximum likelihood in a generalized linear finite mixture model by using the EM algorithm. Biometrics **49**: 227–231.

JANSEN, R. C., 1993b Interval mapping of multiple quantitative trait loci. Genetics **135**: 205–211.

JANSEN, R. C., and P. STAM, 1994 High resolution of quantitative traits into multiple loci via interval mapping. Genetics **136**: 1447–1455.

KNOTT, S. A., and C. S. HALEY, 1992 Aspects of maximum likelihood methods for the mapping of quantitative trait loci in line crosses. Genet. Res. **60**: 139–151.

LANDER, E. S., and D. BOTSTEIN, 1989 Mapping Mendelian factors underlying quantitative traits by using RFLP linkage maps. Genetics **121**: 185–199.

MARTINEZ, O., and R. N. CURNOW, 1992 Estimating the locations and the sizes of the effects of quantitative trait loci using flanking markers. Theor. Appl. Genet. **85**: 480–488.

MCCULLAGH, P., and J. A. NELDER, 1989 Generalized linear models, in *Monographs on Statistics and Applied Probability 37*. Chapman & Hall, London.

PATERSON, A. H., E. S. LANDER, J. D. HEWITT, S. PETERSON, S. E. LINCOLN et al., 1988 Resolution of quantitative traits into Mendelian fac-

tors, using a complete linkage map of restriction fragment length polymorphisms. Nature **335:** 721–726.

RODOLPHE, F., and M. LEFORT, 1993 A multi-marker model for detecting chromosomal segments displaying QTL activity. Genetics **134:** 1277–1288.

STAM, P., 1991 Some aspects of QTL analysis, in *Proceedings of the Eighth Meeting of the Eucarpia Section Biometrics in Plant Breeding.* Brno, July 1991.

TITTERINGTON, D. M., A. F. M. SMITH and U. E. MAKOV, 1985 *Statistical Analysis of Finite Mixture Distributions.* Wiley, New York.

VAN OOIJEN, J. W., 1992 Accuracy of mapping quantitative trait loci in autogamous species. Theor. Appl. Genet. **84:** 803–811.

ZENG, Z.-B., 1993 Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. Proc. Natl. Acad. Sci. USA **90:** 10972–10976.

ZENG, Z.-B., 1994 Precision mapping of quantitative trait loci. Genetics **136:** 1457–1468.